NEW BIOMEDICAL TECHNOLOGIES

Effect of Cytochrome C and Its Derivatives on the Circulation and Oxidative Metabolism of the Ischemized Brain

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The effects of animal and biotechnological cytochrome C and its hemtetradecapeptide in a heme-isomolar dose on the cerebral bloodflow, oxygen and glucose consumption, and pH of the ischemized brain were studied in cat experiments. Cytochrome of both types and hemtetradecapeptide increased the consumption of oxygen and glucose by ischemized brain tissue and somewhat reduced the development of acidosis. The shifts of oxidative metabolism parameters of the ischemized brain correlated with inhibition of the development of postischemic hypoperfusion of the brain.

Key Words: *cytochrome C; cerebral circulation; oxidative metabolism of the brain; brain ischemia*

A study of the antihypoxic and antiischemic effects of biotechnologically prepared cytochrome C (C_b) and its hemtetradecapeptide (HTDP) showed that in terms of these pharmacological characteristics the preparations were similar in activity to cytochrome C of animal origin (C_a) [1]. Later findings demonstrating the protective effect of cytochrome C_b and HTDP in brain ischemia of rats [4] and clinical data on the favorable effect of cytochrome C_a in disorders of cerebral circulation [5,9] underscore the need for further studies in this direction. This paper presents the results of cat experiments investigating the effects of cytochromes C_a, C_b, and HTDP on the total cerebral bloodflow and some parameters of oxidative metabolism in the brain.

MATERIALS AND METHODS

Experiments were carried out with 24 cats of both sexes weighing 2.5 to 3.5 kg narcotized with Nembutal in a dose of 40 mg/kg intraperitoneally. Lung ventilation was carried out using an artificial respiration device with blood gases monitored. The pH, Pco_2, Po_2, and glucose content were measured 30, 60, and 90 min after acute brain ischemia in microsamples of blood. Arterial blood for analysis was collected from the carotid artery by cannulation of one of its branches, while venous blood was taken from the outflow of the venous sinuses.

Oxygen and glucose consumption by brain tissue was calculated from the arterial difference of oxygen and glucose, respectively, with consideration for the volumic velocity of the cerebral bloodflow, which was measured with a device for continuous recording of this parameter [2]. The device was connected to the carotid arteries during ligation of their extracranial branches on both sides. The vertebral arteries were ligated so as to disconnect the perfused area completely from the total arterial system [3].

Systemic arterial pressure (SAP) was recorded by mechanotron sensors [8]. Blood clotting was prevented by intravenous heparin.

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TABLE 1. Effects of Cytochrome C_a (5 mg/kg – I), Cytochrome C_b (5 mg/kg – II), and HTDP (0.8 mg/kg – III) on the Cerebral Bloodflow of Narcotized Cats during the Postischemic Period ($M \pm m$)

Parameter	Initial values	Shifts in % of initial level during ischemia, min		
		30	60	90
Cerebral bloodflow, ml/100 g/min				
control	74.0±2.3	+0.6±4.6	-32.8±4.1^x	-49.4±4.1^x
I	81.4±7.8	+23.4±11.1^x	-19.8±7.7^x	-54.0±5.6^x
II	83.6±2.5	+26.9±7.8*	18.3±8.7^{x*}	-21.5±5.2^{x*}
III	83.6±7.5	+28.4±7.4^{x*}	-5.5±3.0*	-30.6±3.0^{x*}
SAP, mm Hg				
control	104.0±2.2	-16.0±7.3^x	33.9±9.0^x	-37.3±11.6^x
I	115.6±7.2	-22.1±4.7^x	-24.9±8.7^x	-33.9±7.7^x
II	101.9±5.9	-4.0±5.7	+2.1±5.2*	-16.9±7.0^x
III	115.1±10.1	-1.9±2.2	+6.9±3.5^x	-22.5±2.3^x

Note. Here and in Table 2: $p<0.05$: ^{x}*vs.* the initial level, *vs. the control.

Brain ischemia was induced by clamping the carotid arteries and lowering SAP to 40 mm Hg.

During ischemia the clamps were removed from the vertebral arteries to preserve a certain level of blood supply to the medulla oblongata and its functions [6]. Cytochrome C_a and C_b was injected intravenously at the 15th minute of the postischemic period in a dose of 5 mg/kg (reported to cause a stable antiischemic effect in dog experiments). HTDP was injected in a dose isomolar to cytochrome C_b for heme (0.8 mg/kg).

RESULTS

The phase of reactive hyperemia is known to develop after 15-minute deep ischemia of the brain [7]. It is followed by a phase of delayed hypoperfusion, which lasted to the end of the follow-up (120 min) in our experiments. After injection of cytochrome C_b and HTDP, SAP was virtually the same for 60 min as initially. On the other hand, after injection of C_a, SAP gradually and reliably dropped below the initial level, similarly as in the control. By the end of the observation (90 min) SAP in all the experimental series did not differ from that in the control, although the volumic velocity of the cerebral bloodflow under the effect of cytochrome C_b and HTDP reliably differed from the control (its reduction was far less expressed than in the control). This may be due to the capacity of the agents to inhibit the development of postischemic hypoperfusion (Table 1).

In the control, the pH dropped by 2.2-2.6% on average, whereas after cytochrome C_a it fell by no more than 0.8-1.1%. After injection of C_b or HTDP the pH virtually did not differ from the initial value (Table 2).

The time course of oxygen saturation of the blood in the control was characterized by a decrease of arte-

TABLE 2. Effects of Cytochrome C_a (5 mg/kg – I), Cytochrome C_b (5 mg/kg – II), and HTDP (0.8 mg/kg – III) on the Consumption of Oxygen and Glucose and Shifts of Cerebral pH in Narcotized Cats during the Postischemic Period ($M \pm m$)

Parameter	Initial values	Shifts in % of initial level during ischemia, min		
		30	60	90
Oxygen consumption by the brain, ml/100 g/min				
control	3.95±0.21	4.0±9.6	-40.8±12.6^x	-50.3±10.9^x
I	3.78±0.29	+16.8±13.4	-3.0±23.7	-39.0±13.7^x
II	3.03±0.62	+53.5±12.2^x	+0.6±17.6	+9.0±10.4*
III	3.36±0.57	+33.0±10.7^x	+60.0±39.8*	+102.0±21.3*
pH of venous blood				
control	7.290±0.01	-2.2±0.6^x	-2.3±0.7^x	-2.6±0.9^x
I	7.274±0.025	-1.1±0.1^x	-0.8±0.5	-1.0±0.4^x
II	7.220±0.016	-1.8±0.5^x	+0.5±0.2^{x*}	+0.1±0.3*
III	7.190±0.018	-1.0±0.5	-0.2±0.3*	-0.7±0.2^x
Glucose consumption by the brain, μmol/100 g/min				
control	75.3±16.9	-14.0±8.8	-75.0±6.0^x	-51.4±17.1^x
I	60.9±9.4	+80.5±20.2^{x*}	+33.3±19.8*	-34.1±13.5^x
II	101.8±8.3	+72.5±22.5^{x*}	+37.3±17.3^{x*}	-22.3±9.5^x
III	76.5±9.5	+70.4±17.9^{x*}	+6.4±4.3*	-25.0±8.2^x

rial blood saturation and an increase of venous blood saturation. Changes in the oxygen consumption by the brain in these experiments correlated with the changes in the cerebral bloodflow. After injection of cytochrome C_a the saturation of arterial and venous blood with oxygen did not appreciably differ from the initial level, but the calculated value of oxygen consumption by the brain sharply increased during the first 30 min after injection and virtually did not differ from that in the control experiments, despite the drop of the volumic velocity of the cerebral bloodflow. After injection of cytochrome C_b and HTDP an increase of the oxygen consumption by the brain was observed in parallel with a rise of its level in the arterial and a drop in the venous blood, which may be indicative of the functional activity of brain cells. In our experiments cytochrome C and its derivatives did not appreciably alter the content of glucose in the arterial blood in comparison with the control. However, an increase of the cerebral bloodflow during the first 60 min after injection of the drugs led to a reliable increase of glucose consumption by the brain tissue (Table 2).

Hence, cytochrome C_b and HTDP, in addition to inhibiting postischemic hypoperfusion of the brain and reducing postischemic hypotension, restore the glucose and oxygen consumption by the brain deranged during ischemia and alleviate postischemic acidemia. C_b and HTDP exerted the most clear-cut effects on the oxidative metabolism of the brain.

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